

**LETTER TO THE EDITOR**

# Fetal gene therapy for neurodegenerative lysosomal storage diseases

Dear Editor,

For generations of dedicated physicians, neurodegenerative lysosomal storage diseases have been inaccessible to any therapy other than palliative care. Systemic enzyme replacement therapy does not modify the course of the neurological phenotype although improving patients' quality of life by alleviating some symptoms caused by accumulation of biomolecules in peripheral organs. Recent clinical trials are now demonstrating acceptable safety and promising efficacy using various novel therapies including systemic<sup>1</sup> and intrathecal<sup>2</sup> molecules, intracerebroventricular enzyme replacement<sup>3</sup> or intraparenchymal gene therapies.<sup>4</sup> This new hope for these diseases remains elusive for perinatal or infantile presentations when a precipitous clinical decline and subsequent neurological lesions compromise any benefit of currently developed therapies.

For these early-onset presentations, early intervention, perhaps even in utero, may prevent or correct the neurological defect prior to irreversible damage. Liver-directed fetal gene therapy has previously demonstrated long-term expression of transgenic protein up to 6 years after a single injection in non-human primates with adeno-associated viral (AAV) vectors.<sup>5</sup> Theoretical advantages include target of progenitor or stem cells allowing similar effect at reduced dose for integrating vectors<sup>6</sup> and immunological immaturity limiting the risk of immune response against the vector or transgenic protein, which could allow vector re-injection if required.<sup>5</sup> Despite critical unmet needs, there is scant literature describing fetal administration of advanced therapies in animal models of neurodegenerative lysosomal storage diseases.

Massaro et al have exploited AAV-mediated gene therapy to rescue a mouse model of acute neuronopathic Gaucher disease (nGD; or Gaucher disease type II).<sup>7</sup> nGD is caused by glucocerebrosidase deficiency and is at the severe end of a broad phenotypic spectrum of GD,<sup>8</sup> with a reported prevalence of 1:100 000 to 1:300 000 live births.<sup>9</sup> Most affected infants die before the age of 2, following a rapid neurodevelopmental regression with brain stem dysfunction and spastic tetraparesis.<sup>10</sup> A perinatal lethal form presenting

with hydrops fetalis and collodion babies has been described.<sup>11</sup> No effective therapy is available for these infants. The GBA-deficient mouse model reproduces the nGD phenotype with tetraparesis and death before 15 days of age. Neuropathology shows extensive neuroinflammation, neurodegeneration, and accumulation of glucosylceramide and related sphingolipids in brain and peripheral organs.<sup>12</sup>

Massaro et al utilized an AAV9 vector encoding the GBA human cDNA.<sup>7</sup> AAV9 is a neurotropic vector which has shown efficacy after a single systemic injection in a phase I/II trial in infants affected by spinal muscular atrophy. Murine fetal (at day 16 of gestation out of 21) and neonatal intracerebroventricular (ICV) injections showed a marked improvement of the neurological phenotype until 4 months of age, when two out of five animals showed hyperkinesia and stereotypic circling. Neonatal intravenous (IV) injections resulted in normalization of the neurological phenotype and neuropathology findings until termination of the experiment at 6 months. Unlike ICV delivery, IV injections showed clearance of storage phenotype in peripheral organs (liver, spleen, lungs) at 6 months post-injection. Doses of  $5 \times 10^{13}$  vg/kg and  $4 \times 10^{14}$  vg/kg for ICV and IV injection, respectively, were similar to high dose of vectors used in recent clinical trials in infants<sup>13</sup> and adults.<sup>14</sup>

Gene therapy in mouse models of GD has been reported, previously. Both ex vivo lentiviral<sup>15</sup> and in vivo AAV-mediated<sup>16,17</sup> gene therapies have shown an improvement of the visceral phenotype. Massaro et al present, for the first time, a long-term amelioration of GD neuropathology after both central and systemic delivery of gene therapy. A second group recently observed a reduction in GD neuropathology after systemic AAV9 gene therapy.<sup>16</sup> This therapeutic strategy is promising for nGD, for which enzyme replacement therapy is ineffective.

The technical feasibility of ultrasound-guided ICV and IV fetal injections has been reported in macaques<sup>7</sup> and sheep,<sup>18</sup> respectively. The UK Gene Therapy Advisory Committee (GTAC) defined criteria for considering fetal gene therapy, including: (a) clear benefit of fetal compared to post-natal intervention, (b) life-threatening disease with

no therapy.<sup>30</sup> Concerns about germline transmission had been previously raised although this specific risk needs to be balanced with the expected benefit of gene therapy.<sup>19,20</sup> Pre-clinical diagnosis can be performed from fetal DNA in the maternal bloodstream or biopsies of chorionic villi or amniocytes.<sup>8</sup> Antenatal analysis of genotyping<sup>21</sup> and enzymatic studies from biopsies<sup>22</sup> might offer the possibility to better appreciate the severity of the clinical phenotype although phenotypic variability challenges a reliable genotype-phenotype<sup>23,24</sup> and enzymatic assay-phenotype<sup>25</sup> correlations. There are now several new neurotropic AAV variants with enhanced properties for transduction of specific neurological cell-types for example, neurons or astrocytes, or brain areas.<sup>26</sup> These promising achievements may allow the administration of reduced dose of vector for a similar efficacy; this may address recent concerns over possible dose-limited toxicity of AAV9-derived capsids.<sup>27,28</sup>

Fetal gene therapy has remained an elusive goal for decades. This proof of concept study highlights long-term efficacy and technical feasibility of this approach. Clinical translation of this promising technology will require further preclinical steps that is, optimization of the vector construct and studies in large animal models assessing safety for both mother and fetus. This approach could theoretically offer potential treatment or cure for dozens of lethal or severely debilitating infantile neurological inherited metabolic (e.g. molybdenum cofactor and sulphite oxidase deficiencies), or neurogenetic diseases (e.g. monogenic neonatal epileptic encephalopathies), inherited metabolic diseases with acute neonatal decompensations (e.g. urea cycle defects, organic acidurias, some fatty acid oxidation defects), monogenic disorders with early-onset phenotype (e.g. cystic fibrosis with gastrointestinal presentation, surfactant deficiency syndrome), or materno-fetal infection.<sup>29</sup> Rapid progress of novel diagnostics and therapeutics will likely renew interest in prenatal medicine. In turn, fetal medicine may become a preeminent medical specialty as one of the more remarkable advances of the 21st century.

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## CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

## AUTHOR CONTRIBUTIONS

J.B. and S.N.W. jointly planned, conducted and reported the work. J.B. wrote the manuscript. S.N.W. revised the letter. J.B. accepts full responsibility for the content of the manuscript.

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## REFERENCES

- Heron B, Valayannopoulos V, Baruteau J, et al. Miglustat therapy in the French cohort of paediatric patients with Niemann-pick disease type C. *Orphanet J Rare Dis.* 2012;7:36.
- Ory DS, Ottinger EA, Farhat NY, et al. Intrathecal 2-hydroxypropyl-beta-cyclodextrin decreases neurological disease progression in Niemann-pick disease, type C1: a non-randomised, open-label, phase 1-2 trial. *Lancet.* 2017;390:1758-1768.
- Schulz A, Ajayi T, Specchio N, et al. Study of intraventricular Cerliponase alfa for CLN2 disease. *N Engl J Med.* 2018;378:1898-1907.
- Tardieu M, Zerah M, Husson B, et al. Intracerebral administration of adeno-associated viral vector serotype rh.10 carrying human SGSH and SUMF1 cDNAs in children with mucopolysaccharidosis type IIIA disease: results of a phase I/II trial. *Hum Gene Ther.* 2014;25:506-516.
- Mattar CNZ, Gil-Farina I, Rosales C, et al. In utero transfer of adeno-associated viral vectors produces long-term factor IX levels in a Cynomolgus macaque model. *Mol Ther.* 2017;25:1843-1853.
- Karda R, Buckley SM, Mattar CN, et al. Perinatal systemic gene delivery using adeno-associated viral vectors. *Front Mol Neurosci.* 2014;7:89.
- Massaro G, Mattar CNZ, Wong AMS, et al. Fetal gene therapy for neurodegenerative disease of infants. *Nat Med.* 2018;24:1317-1323.
- Stirnemann J, Belmatoug N, Camou F, et al. A review of Gaucher disease pathophysiology, clinical presentation and treatments. *Int J Mol Sci.* 2017;18:441.
- Nalysnyk L, Rotella P, Simeone JC, Hamed A, Weinreb N. Gaucher disease epidemiology and natural history: a comprehensive review of the literature. *Hematology.* 2017;22:65-73.

10. Mignot C, Doummar D, Maire I, De Villemeur TB. French type 2 Gaucher disease study G (2006) type 2 Gaucher disease: 15 new cases and review of the literature. *Brain Develop.* 2006;28:39-48.
11. Finn LS, Zhang M, Chen SH, Scott CR. Severe type II Gaucher disease with ichthyosis, arthrogyposis and neuronal apoptosis: molecular and pathological analyses. *Am J Med Genet.* 2000;91:222-226.
12. Enquist IB, Lo Bianco C, Ooka A, et al. Murine models of acute neuronopathic Gaucher disease. *Proc Natl Sci USA.* 2007;104:17483-17488.
13. Mendell JR, Al-Zaidy S, Shell R, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. *N Engl J Med.* 2017;377:1713-1722.
14. Rangarajan S, Walsh L, Lester W, et al. AAV5-factor VIII gene transfer in severe hemophilia a. *N Engl J Med.* 2017;377:2519-2530.
15. Dahl M, Doyle A, Olsson K, et al. Lentiviral gene therapy using cellular promoters cures type 1 Gaucher disease in mice. *Mol Ther.* 2015;23:835-844.
16. Du S, Ou H, Cui R, et al. Delivery of Gba gene using AAV9 vector therapy as a treatment strategy in mouse models of Gaucher disease. *Hum Gene Ther.* 2018. In Press.
17. McEachern KA, Nietupski JB, Chuang WL, et al. AAV8-mediated expression of glucocerebrosidase ameliorates the storage pathology in the visceral organs of a mouse model of Gaucher disease. *J Gene Med.* 2006;8:719-729.
18. Themis M, Schneider H, Kiserud T, et al. Successful expression of beta-galactosidase and factor IX transgenes in fetal and neonatal sheep after ultrasound-guided percutaneous adenovirus vector administration into the umbilical vein. *Gene Ther.* 1999;6:1239-1248.
19. Couzin J. RAC confronts in utero gene therapy proposals. *Science.* 1998;282:27.
20. Moulton G. Panel finds in utero gene therapy proposal is premature. *J Natl Cancer Inst.* 1999;91:407-408.
21. Yoshida S, Kido J, Matsumoto S, et al. Prenatal diagnosis of Gaucher disease using next-generation sequencing. *Pediatr Int.* 2016;58:946-949.
22. Gort L, Granell MR, Fernandez G, Carreto P, Sanchez A, Coll MJ. Fast protocol for the diagnosis of lysosomal diseases in nonimmune hydrops fetalis. *Prenat Diagn.* 2012;32:1139-1142.
23. Goker-Alpan O, Hruska KS, Orvisky E, et al. Divergent phenotypes in Gaucher disease implicate the role of modifiers. *J Med Genet.* 2005;42:e37.
24. Gupta N, Oppenheim IM, Kauvar EF, Tayebi N, Sidransky E. Type 2 Gaucher disease: phenotypic variation and genotypic heterogeneity. *Blood Cells Mol Dis.* 2011;46:75-84.
25. Beutler E, Grabowski GA. Gaucher disease. *The Metabolic and Molecular Bases of Inherited Disease.* New York, NY: McGraw-Hill; 2001:3635-3668.
26. Hammond SL, Leek AN, Richman EH, Tjalkens RB. Cellular selectivity of AAV serotypes for gene delivery in neurons and astrocytes by neonatal intracerebroventricular injection. *PLoS One.* 2017;12:e0188830.
27. Hinderer C, Katz N, Buza EL, et al. Severe toxicity in nonhuman primates and piglets following high-dose intravenous administration of an adeno-associated virus vector expressing human SMN. *Hum Gene Ther.* 2018;29:285-298.
28. Hordeaux J, Wang Q, Katz N, Buza EL, Bell P, Wilson JM. The neurotropic properties of AAV-PHP.B are limited to C57BL/6J mice. *Mol Ther.* 2018;26:664-668.
29. Coutelle C, Douar AM, Colledge WH, Froster U. The challenge of fetal gene therapy. *Nat Med.* 1995;1:864-866.
30. Gene Therapy Advisory Committee. Report on the potential use of gene therapy in utero. Health departments of the United Kingdom. 1998. *Hum Gene Ther.* 10:689-692.